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ŀ	(54) Title: METHOD FOR ACCELERATING GR	OWT		ATEG	

(54) Title: METHOD FOR ACCELERATING GROWTH RATES

(57) Abstract

Upon subjection to a low energy electrical field of varying and critical frequencies and low field strengths the growth rate of an organism disposed in a growth medium exhibits a significant increase over power-off controls.

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METHOD FOR ACCELERATING GROWTH RATES

1. Field of the Invention

This invention relates to a method for increasing the growth rate of biosystems. More particularly, this invention provides a method for accelerating the growth rates of organisms which involves the use of low energy electrical fields produced by a unique combination of applied low field strengths and extra-low electromagnetic frequencies.

Prior Art

- 10 The effects of electrical fields on the stimulation of growth and regeneration in various biosystems have been studied. Mcelhaney et al., J. Biomech. 1:47 (1968) disclose the use of electrical fields to stimulate bone formation. The use of applied magnetic
- fields to accelerate growth in microorganisms is shown 15 in U. S. Patent 3,871,961. Sewage decomposition promoting microorganisms are stated in U. S. Patent 3,336,220 to exhibit increased activity after an alternating electric current was passed through the sewage.
- 20 MacKenzie in New Scientist, 28 January 1982, pp. 217-220 reports on electrical fields which develop in connection with loss of limbs and which are implicated in the resultant regeneration phenomena. The effects of electrical fields on movement in an amoeba was reported in Science, <u>187</u>:357 (1975). 25

Much of the work performed studying the effects of electrical fields on microorganisms has involved the study of mutagenesis and also sterilization, e.g., see Coate, W. B. et al. 1970, "Project Biological Effects Test Program Pilot Studies Final Report. " Hazilton Laboratories, Inc., available from NTIS as AD717409,

U. S. Patent 3,876,373 and Food Tehnol, 8:361 (1954).

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It is an object of this invention to provide a unique and practical methodology utilizing low energy electrical fields to accelerate growth rates when applied to a living biosystem. It is a further object of this invention to increase such growth rates without producing mutants and without a measurable increase in the temperature of the biosystem. It is a still further object of this invention to provide an energy efficient, low cost and effective process for significantly reducing the growth cycle time of a biosystem.

DESCRIPTION OF THE INVENTION

These and the other objects of this invention are accomplished by a process for accelerating the growth rate of a live organism which comprises disposing the organism in a suitable growth or nutrient medium and 15 subjecting the organism to an alternating electrical field at an electrical field strength and frequency combination which causes the growth rate of the organism to increase over the growth rate of the organism when not subjected to the alternating electrical field. 20 The desired increase in growth rate is independent of current density and is exhibited at varying frequencies and electrical field strengths. However, as far as has been tested there appear to exist certain critical fre-25 quencies, or regions of frequencies, at which accelerated growth occurs and outside of which there is minimal or no effect. Also at such effective frequencies there exists a threshold electrical field strength below which there is no effect. It has also been found that, within the ranges of effective frequencies and 30 field strengths, the resultant electrical field is a low energy field which does not produce mutants of the organism; nor is there a measurable increase in the temperature of the biosystem. Furthermore, in the preferred ranges of effective frequencies and strengths 35 there is no electrolysis of the growth medium. It has

15

found that the effective frequencies and voltages are independent of current density. However, there is a practical limitation on current density in that one would not employ currents that adversely affect the biosystem. Tests have been conducted at less than 0.1 mA (milliamp) and it is believed that absolutely no current is required to effectively increase growth rates, so long as effective field strengths and frequencies are utilized in carrying out the invention.

10 Definition of Terms

"Growth Rate (rate)" - This is obtained by taking the Klett units over time and calculating the doubling time for the culture. This is done by plotting the Klett unit number of each reading on the log scale of a semilog graph (wherein the y axis is the semi-log scale and the x axis is time). The slope (m) of the curve is then calculated using the following formula:

$$m = \frac{E_{xy} - \frac{E_{x}E_{y}}{N}}{E_{x}^{2} - \frac{(E_{x})^{2}}{N}}$$

wherein m is the slope, x is time, y is the semi-log, and N is the number of individual Klett readings.

"Klett units" - a unit of measurement of optical density employing a Klett-Summerson photoelectric colorimeter.

"LeeGs value (L)" is obtained by dividing the growth
rate of the test sample by the growth rate of the
control sample thus

$$L = \frac{G \text{ test}}{G \text{ cont}}$$

"Correlation (coor)" is calculated in accordance with following mathematical sequence:

$$corr = R = \frac{Ms_x}{s_y}$$

wherein s is the standard deviation of the x and y axes of the semi-log graph referred to in the definition of growth rate.

"Dev" is an abbreviation for the standard deviation between the control groups calculated in the following manner:

10 Dev =
$$\left(\frac{\operatorname{sum}(x^2) - \left[\frac{\operatorname{sum}(x)}{N} \right]^2}{N-1} \right)^{\frac{1}{2}}$$

wherein x is the value of the growth rate of each control group and n is the number of control groups.

"L-broth" - a growth medium consisting of 0.8% NaCl, 0.8% glucose and 0.7% bacto-tryptone, all percentages being weight to volume of tap water.

"Biosystem" - a system comprised of the organism disposed in a medium suitable for its normal growth.

"Hz" - abbreviation for hertz, a unit synonomous for cycle per second.

20 "KHz" - 1 kilohertz or 1000 hertz.

"ATCC" - American Type Culture Collection a depository for microorganisms located in Rockville, Maryland.

Example 1

An overnight exponential culture of prototropic E.

coli K-12 (Yale University depository number CGSC No. 4401) growing at 37°C, in L-broth was diluted with thermally equilibrated and sterilized L-broth to a density of 1×10^6 cells per milliliter. Portions of this culture were then randomly placed into 10 ml. glass test tubes made of the type glass specified for the colorimeter employed. Each test tube contained two platinum electrodes, spaced 4 centimeters apart, wired in series to a variable frequency generator and in 10 parallel to an oscilliscope, and equipped with means for oxygen input to the growth medium. The tubes containing the fractionated culture medium were maintained at constant temperature in a thermostatically controlled water bath and each subjected to an electromotive (EmF) field. Temperature was monitored with a 15 glass/mercury thermometer. Each tube was isolated from ~ the field of the other tubes by brass screening. This brass screening was grounded to totally eliminate any electrical transfer from one tube to the other. The 20 culture densities of each test tube were tested with a Klett-Summerson photoelectric colorimeter by measuring, at 20-30 minute intervals over a 3 hour period, increasing optical absorbences at 660 nanometers. Controls were also run using cells that were inac-25 tivated by subjecting them momentarily to 60°C and quick cooling to 37°C. Each set of controls consisted of three test tubes which were measured at 10, 100 and 1000 hertz to establish the background of optical absorbence effects due to the electroplating of cells 30 and growth medium components onto the electrodes and also to ascertain any electrolytic effects that might be present. The EmF fields were produced by the variable frequency generator and monitored via the oscilliscope. The low energy fields were applied by

passage of alternating current through the growth medium between the platinum electrodes.

The results obtained with \underline{E} , $\underline{\operatorname{coli}}$, following the above procedure are set forth in the following tables.

Table 1

At 37°C, 1KHz, 10V.cm

time	0	20	40	60	80	rate	coor
con exp	37 37	39 41	42 45	48 58	52 53	0.37	0.99

1.27

Table 2

At 34°C, 1KHz, 18V/cm

	time	0	30	60	rate	
15	conl	66	68	72	0.12	0.98
	con2	50	52	55	0.14	0.99
	expl	49	53	58	0.24	1.00
	exp2	93	98	105	0.17	1.00

cont avg. = 0.13, dev = 0.01L1 - 1.85, L2 - 1.31

20

Table 3

At 37°C, 10KHz, 5V/cm

	time	0	30	60	rate	coor
25	conl	65	76	88	0.44	1.00
	con2	65	78	89	0.46	1.00
	expl	71	84	99	0.48	1.00
	exp2	66	81	98	0.57	1.00

cont avg. = 0.45, dev = 0.01 L1 - 1.07, L2 - 1.27

. Table 4
At 37°C, power off

	time	60	90	rate	coor
5	conl	88	107	0.56	1.00
	con2	89	108	0.56	1.00
	expl	99	112	0.36	1.00
	exp2	98	112	0.39	1.00

cont avg. = 0.56, dev = 0.00L1 - 0.64, L2 - 0.70

10

Table 5

At 37°C, 1KHz, 5V/cm

	time	120	150	rate	coor
15	conl con2 expl exp2	141 130 137 135	154 148 151 151	0.25 0.37 0.28 0.32	1.00 1.00 1.00

cont avg. = 0.31, dev = 0.08L1 - 0.90, L2 - 1.03

Table 6

20 At 37°C, 1KHz, 5V/cm

	time	0	10	95	110	rate	coor
25	conl	62	64	91	94	0.34	1.00
	con2	58	61	89	95	0.42	1.00
	expl	77	82	123	117	0.79	0.98
	exp2	64	68	94	95	0.65	0.99

cont avg. = 0.38, dev = 0.06 L1 - 2.08, L2 - 1.71

Table 7
At 36°C, 100Hz, 5V/cm

	<u>time</u>	0	30	60	rate	coor
5	conl	133	138	153	0.20	0.96
	con2	140	144	152	0.12	0.98
	expl	135	139	152	0.17	0.96
	exp2	144	149	159	0.14	0.98

cont avg. = 0.16, dev = 0.06L1 - 1.06, L2 - 0.88

10

Table 8

At 37°C, 1KHz, 10V/cm

	time	0	30	60	90	120	rate	coor
15	conl con2 expl exp2	120 132 128 125	135 141 141 137	153 158 164 160	174 179 185 180	200 200 200 200 200	0.36 0.30 0.34 0.35	1.00 1.00 1.00

cont avg. = 0.33, dev = 0.04L1 - 1.03, L2 - 1.06

Table 9

20

At 34°C, 500Hz, 5V/cm

	time -	0	30	60	rate	coor
25	conl con2 expl exp2	132 129 121 112	144 145 136 120	158 155 146 133	0.26 0.27 0.27 0.25	1.00 0.99 0.99

cont avg. = 0.27, dev = 0.01L1 = 1.00, L2 = 0.93

 $\frac{\texttt{Table 10}}{\texttt{At 40°C, 5KHz, 5V/cm}}$

	time	0	30	90	rate	coor
5	conl	108	119	142	0.26	1.00
	con2	107	113	139	0.25	0.99
	expl	107	121	140	0.26	0.99
	exp2	113	126	142	0.22	0.99

cont avg. = 0.26, dev = 0.01L1 = 1.00, L2 = 0.85

10

Table 11

At 37°C, 1KHz, 8V/cm

	<u>time</u>	0	30	60	90	120	rate	coor
15	conl	98	103	106	115	122	0.16	0.99
	con2	103	105	109	116	125	0.14	0.97
	expl	95	100	109	120	122	0.20	0.98
	exp2	95	102	115	118	120	0.18	0.95

cont avg. = 0.15, dev = 0.01L1 = 1.33, L2 = 1.20

Table 12

20

At 37°C, 100Hz, 5V/cm

time	0	70	90	rate	coor
conl	107	127	129	0.19	0.99
con2	93	111	115	0.21	1.00
expt	95	116	113	0.19	0.94

25 cont avg. = 0.20, dev = 0.01, L = 0.95

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Table 13
At 37°C, lKHz, 3V/cm

	time	. 0	70	90	rate	coor
5	conl	107	127	129	0.19	0.99
	con2	93	111	115	0.21	1.00
	expt	84	100	107	0.23	1.00

cont avg. = 0.20, dev = 0.01, L = 1.15

Table 14

At 37°C, 10KHz, 5V/cm

10	time	00	30	60	rate	coor
	conl	33	34	37	0.16	0.96
	con2	33	34	3 7	0.16	0.96
	expt	33	34	39	0.23	0.94

cont avg. = 0.16, dev = 0.00, L = 1.44

Table 15

15 At 37°C, 1KHz, 4V/cm

time	0	30	60	rate	coor
conl	33	34	37	0.16	0.96
con2	33	34	37	0.16	0.96
expt	38	42	48	0.33	1.00

20 cont avg. = 0.16, dev = 0.00, L = 2.06

 $\frac{\text{Table 16}}{\text{At 35°C, 1KHZ, 4V/cm}}$

	time	0	30	60	90	120	rate	coor
5	conl	36	41	44	48	54	0.28	1.00
	con2	39	44	50	62	79	0.48	0.99
	expt	39	45	59	78	103	0.67	0.99

cont avg. = 0.38, dev = 0.14, L = 1.76

Table 17

At 34°C, 1KHz, 2V/cm

10	time	0	30	60	90	120	rate	coor
	conl	36	41	44	48	54	0.28	1.00
	con2	39	44	50	62	79	0.48	0.99
	expt	44	47	53	60	65	0.29	1.00

cont avg. = 0.38, dev = 0.14, L = 0.76

15

Table 18

At 37°C, .0Hz, 4V/cm

	time	0	30	60	85	rate	coor
20	conl	42 .	43	44	42	0.01	0.18
	con2	37	38	39	34	-0.07	-0.46
	expt	35	48	55	70	0.70	0.99

Table 19

1KHz, 1V, 0.4% NaCl

cime	0	46	61	<u>75</u> .	rate	coor
cont expt	23		34 36		0.49	

(Power was inadvertently on on the control at lKHz., 0.5V until the time of the 46 minute reading, therefore the valid reading would start at that time. Starting

from 46 minutes the results turn out that the growth rate of the control turns out to be 0.45 gen/hr with a correlation of 0.99 and the rate of the experimental is 0.90 gen/hr with 1.00 correlation. This gives L=2.00 which corresponds with previous data.)

Table 20

Testing of 0.4 & 0.2 NaCl to show that the effect is not due to current.

1KHz., 1V 10 time 0 30 60 70 80 90 rate coor 0.4% 20.0 22.0 25.0 27.5 29.5 32.0 0.43 0.98 0.2% 9.0 10.0 10.0 11.5 12.5 14.0 0.36 0.91 power off time 0 30 60 rate coor 15 0.4% 32.0 35.5 39.5 0.29 0.90 0.2% 14.0 15.0 17.0 0.28 0.99 1KHz., 1V time 0 10 20 30 rate coor 0.4% 39.5 42.5 47.5 49.0 0.66 0.98 20 0.2% 17.0 21.0 22.5 24.5 1.07 0.96 0.4% rate avg. = 0.54, div = 0.16, L = 1.860.2% rate avg. = 0.72, div, div = 0.50, L = 2.57

That mutagenesis was not implicated in the acceleration of growth rates for <u>E. coli</u> was demonstrated by presence of carbon dioxide evolution when a culture medium from Example 1 was added to a test tube containing lactose and mineral salts at the temperature of 43°c (Eijkmann-Durham Test for contaminants).

Example 2

The procedure of Example 1 was followed except that the microorganism was $\underline{Bacillus}$ subtilus (ATCC 6051). The following results were obtained:

power off (control) temp. = 22c

time	0	30	60	rate	coor
A	0.126	0.132	0.145	0.200	0.980
B	0.028	0.033	0.036	0.370	

power lKHz, lV/cm

10	time	0	30	60	90	rate	coor
	A				0.206		
	В	0.036	0.041	0.053	0.064	0.553	0.993

A L = 1.68; B L = 1.50 L = 1.59

Example 3

The procedure of Example 1 was employed except that 2% Bacto yeast extract (Fleichman's fresh active yeast) was employed in place of <u>E. coli</u> and was grown at 25°C in an aqueous solution of 0.3% Bacto-Tryptone and 4.0% glucose (percentages are weight to volume water). The following results were obtained:

power off (control)

	time	0	60	. 1:	20	rate	coor		
	1 2	67 99	69 100	71	.5 05	0.05 0.04	1.00		
25	lKHz., lV/cm								
	time	0	60	120	180	rate	coor		

 time
 0
 60
 120
 180
 rate
 coor

 1
 71.5
 75
 81
 83
 0.08
 0.98

 2
 105
 110
 113
 118
 0.05
 1.00

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+ i ma

power off (control)

60

	time	<u> </u>	6	0	rate	coor
	ch-1 ch-2	83 118	8 12	_	0.03	1.00
5			power 100 H	z., lV/cm.		
	time	0	60	100	rate	coor
	1 2	85 120	87.5 123	89 124	0.04 0.03	1.00
10		cont	ch-1 avg = 6 ch-2 avg = 6 ch-1 1KHz.	0.03, dev =	= 0.01	
			ch-2 1KHz. = L 1KHz. av ch-1 100 Hz	vg = 1.84		

Example 4

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This example demonstrates the efficacy of the process of the invention in accelerating plant growth rates.

rate $ch-2\ 100\ Hz. = 0.03$, L = 1.00

- Glass or plastic containers containing soil and bean seeds (Bountiful Green Bush variety of Pinto) were configured for generation of low energy electrical fields in the following four modes:
- parallel brass screens above and below the soil
 between which the beans are positioned without touching the screens
 - 2. one brass screen parallel to the side of the container with the seeds spaced apart therefrom with intervening soil in between the seeds and the screen
- 30 3. parallel wires in the horizontal plane of the bean seeds and spaced apart therefrom

one brass screen at the bottom of the container below the bean seeds.

Each container had from 4-8 bean seeds planted therein. All wires and screens were connected in series to an alternating current generator capable of producing multiple functions (e.g., sine or square waves). For each planted container connected to the generator there was a power-off control container of the same configuration. All other conditions were the same for

10 controls and test groups. To each test container were applied 22 volts (approximately 2.5 volts/cm.) at 1 KHz over a period of one week. At the end of the test period the test groups averaged about 8.5 inches in height, whereas the controls had just commenced to ger-

15 minate. The test group had significantly higher germination success than the controls. At comparable stages of development the test group was more vigorous in appearance than the controls and hence appeared to exhibit an increase in developmental success.

20 Example 5

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Into a glass container was added a brass screen upon which was placed fruit fly medium containing freshly laid eggs. On top of the medium was placed another screen which along with the screen on the bot-25 tom of the container, was connected in series with a variable frequency generator. To the test sample was applied a field of lKHz, 22V (about 2.5 V/cm.); the control was a power off sample. The flies (Drosophila melanogaster) were then observed to eclosure (reaching of adulthood), and observed to show an earlier eclosure in the test sample than the control. The test sample also exhibited a greater hatching and eclosure success

and also was more vigorous (i.e., observably significantly more active than the control.)

The foregoing examples demonstrate that 1000 Hz and 10,000 Hz are highly preferred effective frequencies for growth rate acceleration with widely differing spe-5 cies of organisms at field strengths, which can be expressed in terms of volts per centimeter, ranging upwards of from about 1 volt per centimeter to about 22 volts per centimeter at the expressed frequencies. 10 However, positive growth rate acceleration can be achieved at selected frequencies ranging upwards from 10 Hz to about one megahertz (MH $_{\mathbf{Z}}$), the only practical limitation being that point at which mutagenesis occurs. A wide range of field strengths can be employed at each selected effective frequency with a 15 concomitant threshold field strength below which no effect occurs. Again practicality dictates that the upper limit for such field strengths is that point at which a measurable temperature increase occurs in the 20 biosystem. Selection of these critical frequencies and field strengths can routinely be accomplished by performing a simple series of tests as follows:

field is applied across the biosystem, to which electrodes are applied selected frequencies at a set minimal field strength of lV/cm. Upon discovery of an effective frequency for growth rate increase, various field strengths are applied at the set effective frequency to determine the effective field strength

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range. With microorganisms it is recommended that the system be designed essentially as described in Example 1. With plant or animal species any one of the systems described in Example 4 can be utilized. If no effective frequency is found at 1 volt/cm., repeat procedure at 5 volts/cm. and at 5 volt increments thereafter until an effective growth rate increasing frequency is found or until a measureable temperature increase occurs in the biosystem using a standard glass mercury thermometer.

Increase of growth rates while improving developmental success and vitality of some organisms in accordance with this invention has important commercial implications for industrial fermentation, as well as, agricultural and pharmaceutical production industries.

Improved yields and increased production capacities are direct advantages. Shorter organism growth cycle could open new vistas in diagnostic medicine as well as other areas of medicine such as bone marrow regeneration, organ regeneration, tissue revitilization and the like.

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What is claimed is:

- 1. A method of increasing the growth rate of a living organism which comprises disposing the organism in a growth medium and subjecting the organism to a low energy electrical field produced by a combined field strength and frequency effective for increasing the growth rate of said organism.
- The method of claim 1 wherein said field strength is above about 1 volt per centimeter and said
 frequency is selected from about 10 to about 10⁶ Hz.
 - 3. The method of claim 1 wherein said organism is a microorganism or plant or animal.
- 4. The method of claim 3 wherein said voltage is above about 1 volt per centimeter and said frequency is selected from about 1000 Hz and about 10,000 Hz and the resulting electrical field does not increase the temperature of the medium.
- 5. The method of claim 3 wherein said voltage is selected from above about 1 volt per centimeter to about 22 volts per centimeter and said frequency is about 1000 Hz or about 10,000 Hz.

INTERNATIONAL SEARCH REPORT

International Application NoPCT/US 86/02271

According to International Patent Classification (IPC) 47/9	ral classification symbols apply, Indicate all) 3 both National Classification and IPC 4; AUTK 69/000	
U.S. C1: 435/173; 426/2; 47	7/1.3; 119/1	
II. FIELDS SEARCHED		
Classification System	Documentation Searched 4	
	Classification Symbols	· · · · · · · · · · · · · · · · · · ·
U.S. 435/173,243;426/	2;47/1.3;119/1	
Documentation Searche to the Extent that such Do	d other than Minimum Documentation cuments are included in the Fields Searched 6	
III. DOCUMENTS CONSIDERED TO BE RELEVANT	14	
Category • Citation of Document, 16 with indication, wh	nere appropriate, of the relevant passages 17	Relevant to Claim No. 18
$\frac{X}{Y}$ US, A, 3,336,220 (No. 1967, See col. 2, 1) col. 3, lines 5-20 and col. 6, lines 2	lines 15-20, and 45-50	$\frac{1,3}{2,4,5}$
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 Special categories of cited documents: 13 "A" document defining the general state of the art which is considered to be of particular relevance "E" earlier document but published on or after the internatifiing date "L" document which may throw doubts on priority claim(s which is cited to establish the publication date of ancitation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition other means "P" document published prior to the international filing date later than the priority date claimed IV. CERTIFICATION 	invention "X" document of particular relevant cannot be considered novel or involve an inventive step document of particular relevant cannot be considered to involve a document is combined with one ments, such combination beloss.	ct with the application but or theory underlying the es; the claimed invention cannot be considered to es; the claimed invention in inventive step when the or more other such docu- byious to a person skilled
Pate of the Actual Completion of the International Season	Date of Mailing of this International Sec	arch Popert
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A	US, A, 2,492128 (SHROPSHIRE), 20 December 1949, see entire document.	
	ERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE 10	
This intern	tional search report has not been established in respect of certain claims under Article 17(2) (a) fo numbers, because they relate to subject matter 12 not required to be searched by this Aut	r the following reasons: thority, namely:
2. Claim ments	numbers, because they relate to parts of the international application that do not comply w to such an extent that no meaningful international search can be carried out ¹³ , specifically:	ith the prescribed require-
	RVATIONS WHERE UNITY OF INVENTION IS LACKING 11	
This Internat	onal Searching Authority found multiple inventions in this international application as follows:	
	•	
1. As all r	equired additional search fees were timely paid by the applicant, this international search report cov ternational application.	ers all searchable claims
2. As only	some of the required additional search fees were timely paid by the applicant, this international salms of the international application for which fees were paid, specifically claims:	
3. No requ the inve	ired additional search fees were timely paid by the applicant. Consequently, this international search flow first mentioned in the claims; it is covered by claim numbers:	ch report is restricted to
Remark on Pr		erching Authority did not
The add	tional search fees were accompanied by applicant's protest.	
☐ NO brot	st accompanied the payment of additional search fees.	

Form PCT/ISA/210 (supplemental sheet (2)) (October 1981)

III. DOCUM	PCT ENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHE	/US 86/02271
Category * j	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	
	more appropriate, of the relevant passages to	Relevant to Claim No
!		
A .	US, A, 3,871,961 (GIANESSI), 18	
:	March 1975, see entire document.	:
A ;	US, A, 4,487,834 (BRIGHTON), 11	· <u>:</u>
•	December 1984, see entire document.	į
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